

CROP BREEDING, GENETICS & CYTOLOGY

Divergent Selection for Rind Penetrometer Resistance and Its Effects on European Corn Borer Damage and Stalk Traits in Corn

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ABSTRACT

Corn (*Zea mays* L.) grain yield is affected by a number of factors, including stalk lodging and pests such as the European corn borer (*Ostrinia nubilalis* Hübner; ECB). European corn borers contribute to stalk lodging and also cause a direct grain yield reduction through physiological effects that decrease the plant's ability to produce and translocate photosynthates. Although much progress has been made in improving standability, stalk lodging remains a major problem, and breeding for stalk lodging resistance continues to be important, especially if it also plays a role in ECB resistance. Missouri Second Cycle Stiff Stalk Synthetic (MoSCSSS) was selected for stalk strength by using a rind penetrometer. Twelve cycles of bidirectional selection have been completed, which has resulted in increased and decreased stalk strength in the high and low directions of selection, respectively. Selected cycles were evaluated for grain yield, stalk lodging, rind penetrometer resistance, first- and second-generation ECB damage, leaf penetrometer resistance at the whorl stage and anthesis, and stalk traits including crude fiber, cellulose, lignin, and silica. Evaluation showed a decrease in grain yield in both directions of selection. Selection for high rind penetrometer resistance was effective at providing resistance to second-generation ECB damage as well as resistance to stalk lodging. Leaf penetrometer resistance was higher in the high direction of selection at whorl stage, but reversed by anthesis where the low direction of selection had higher leaf penetrometer resistance. Crude fiber, cellulose, and lignin increased in the high direction of selection, but silica decreased in the high direction of selection. Significant correlations between the stalk traits analyzed demonstrated that stalk composition was important in providing rind penetrometer resistance, stalk lodging resistance, and second-generation ECB resistance.

CORN GRAIN YIELD is affected by a number of factors, including stalk lodging and pests such as the ECB. Stalk lodging accounts for 5 to 25% annual grain yield losses in the USA (Zuber and Kang, 1978). European corn borers contribute to stalk lodging and also cause a direct grain yield reduction through physiological effects that decrease the plant's ability to produce and translocate photosynthates. In addition to stalk lodging, ECB infestations also indirectly affect the incidence of ear droppage and the spread of stalk- and ear-rotting organisms, all of which contribute to grain yield losses (Jarvis et al., 1982; Klenke et al., 1986).

Although much progress has been made in improving standability, stalk lodging remains a major problem, and breeding for stalk lodging resistance continues to be

important, especially if it also contributes to ECB resistance. Increased stalk strength could increase the resistance to ECB and provide farmers nontransgenic germplasm with greater resistance than is available to date. Additionally, with the 20% refuge mandate of nontransgenic varieties when using Bt-corn hybrids, it is important to continue to increase the quality of corn within this category.

Much of the strength of a corn stalk comes from the outside portion, the rind. Rind strength can easily be measured as the force required to puncture the rind with a needle with a rind penetrometer (Sibale et al., 1992; Chesang-Chumo, 1993; Masole, 1993). Selections have been made for rind penetrometer resistance in both the high and low directions in MoSCSSS (Gerdes et al., 1993). The low cycles decrease in rind strength and the high cycles increase in rind strength. An increase in stalk strength may allow the plant to better deter and withstand the direct and indirect effects of ECB feeding damage.

Previous research with MoSCSSS has shown a correlation of improved stalk lodging resistance and increased rind penetrometer resistance. Chesang-Chumo (1993) showed that rind penetrometer resistance measurements were highly correlated with stalk lodging resistance, and there was a proportional decrease in stalk lodging with high rind penetrometer resistance. Masole (1993) showed that rind penetrometer resistance measurements were correlated with rind thickness and that the stalk circumference decreased as rind penetrometer resistance increased.

Zuber et al. (1980) demonstrated that rind thickness was not the only contributing factor to stalk strength and that rind composition may be as important, if not more so. Plant strengthening components like fiber, lignin, cellulose, and silica may affect ECB feeding, both nutritionally and physically. These compounds are found throughout the leaves and the stalk. An increase in concentration of these compounds may reduce digestibility and plant material intake (Buendgen et al., 1990). These strengthening compounds may also play a role in leaf toughness and ECB feeding on the leaves. A study by Bergvinson et al. (1994) found a significant inverse relationship between leaf toughness and ECB leaf-damage ratings at the midwhorl and tasseling stages of plant development. They also found an increase in leaf toughness with maturity.

Rind thickness and stalk strength have a significant impact on stalk lodging, but the impact on ECB feeding has not been assessed. This study addresses (i) whether

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rind penetrometer resistance selection affects second-generation ECB feeding, (ii) whether rind penetrometer resistance selection affects leaf toughness and first-generation ECB feeding, (iii) how rind penetrometer resistance selection changes relative amounts of strengthening compounds in the stalk and their correlation with ECB feeding, and (iv) how 12 cycles of rind penetrometer resistance selection in the high and low directions affects stalk lodging, grain yield, and other agronomic traits.

MATERIALS AND METHODS

Missouri Second Cycle Stiff Stalk Synthetic is a yellow endosperm, dent maize population formed from 14 inbred lines: A657, A632Ht, B14AHt, B37Ht, B68, B73, B76, B84, CM105, H84, N28Ht, N104, Oh514, and Pa864P. These lines were either directly developed from various cycles of Iowa Stiff Stalk Synthetic (BSSS) or were related versions of original BSSS lines.

The parental lines were initially intercrossed, obtaining 84 crosses out of 91 possible combinations for F_1 crosses. These crosses were then planted in isolated blocks containing two replications of each F_1 cross. Rows of crosses were detasseled to allow for pollination with male plants consisting of an aliquot mixture of seed from each F_1 -cross family. An equal number of ears were selected from each replication of each F_1 -cross family at harvest. The same procedure was used for an additional two generations, after which the synthetic was considered formed.

The original synthetic (C0) was used to develop the high and low rind penetrometer resistance populations using S_0 phenotypic recurrent selection. To establish the high and low rind penetrometer resistance populations, prepollination selection was done. First, potential top ears were shootbagged on all plants to protect them from possible outcrossing that might occur before selection. Second, noncompetitive plants were cut off at approximately 50% pollen shedding. These include the end plants on each row and plants on either side of a stunted plant or a missing plant. Selection was made from approximately 600 competitive plants. Rind penetrometer resistance measurements were taken from the remaining competitive plants. A 120-plant sample was used to determine the cut-off points for high and low rind penetrometer resistance in the two populations. Individual plant rind penetrometer resistance readings for about 600 competitive plants in each population were then taken. Nonselected plants were cut off, and the remainder recombined using a bulked-pollen method with an approximate ratio of one male for each two female plants set up. Approximately 120 plants were pollinated for each cycle. The harvest from these pollinated selections formed the subsequent cycle for selection.

Missouri Second Cycle Stiff Stalk Synthetic, which had undergone 12 cycles of selection for high and low rind penetrometer resistance, was evaluated for grain yield, stalk lodging percentage, rind penetrometer resistance, ECB leaf-feeding and tunneling damage, leaf toughness, and percentages of crude fiber, cellulose, lignin, and silica in the stalk. The ECB-resistant and susceptible checks, Pioneer Brand 3184 (Pioneer Hi-Bred International, Inc., Des Moines, IA)¹ and Wf9 × 182E, respectively, were also included. The experiment con-

sisted of 12 entries (Table 1) in a three replication, randomized complete block design grown in six Missouri environments in 2000. Duplicate entries of C0 were included in each replication because of the bidirectional selection for rind penetrometer resistance.

Environments were: Hinkson Bottom, Columbia, MO, early and late plantings on Freeburg silt loam soil (fine-silty, mixed, superactive, mesic Aquic Hapludalf); Bradford Research and Extension Center, 10 km east of Columbia on Mexico silt loam soil (fine, smectitic, mesic Aeric Vertic Epiaqualf); Lee E. Greenley Memorial Research Center, Novelty, MO, on Putnam silt loam soil (fine, smectitic, mesic Vertic Albaqualf); South Farm, 1 km east of Columbia on Mexico silt loam soil; and Marshall, MO, on Sibley silt loam soil (fine-silty, mixed, superactive, mesic Typic Argiudolls).

Plantings at Hinkson Bottom were staggered to create different environments at the same location. Experiments were machine planted with 32 kernels row⁻¹, spaced 19.1 cm apart. Rows were spaced 0.91 m apart and were 6.1 m long. Plots were thinned to 26 plants row⁻¹ approximately 1 mo after planting, when stand counts were also recorded.

Five-row plots were used. Rows 1 and 2 were used for grain yields and moistures, stand counts, and root and stalk lodging counts; Row 3 was used for first- and second-generation ECB damage evaluation; Row 4 was used for rind penetrometer and leaf penetrometer resistance measurements; and Row 5 was for stalk sample analyses. Grain yield and moisture, and root and stalk lodging percentages were recorded on a plot basis at harvest.

Rind penetrometer resistance was measured approximately 10 d after flowering in the middle of the internode below the primary ear attachment for 10 competitive plants in each plot (Fig. 1). The rind penetrometer was a modified AccuForce Cadet digital force gage, 22.7 kg capacity, powered by a 9-V alkaline battery (Ametek, Hunter Division, Hatfield, PA). The modification involved fabrication of a 5-cm shaft to which a needle (20.6 mm long, 2.8-mm diam., tapering to a point across 14.3 mm) was affixed, and attachment of a stop-bar at a 90° angle to the needle axis, just past the needle taper area. Rind penetrometer resistance was measured as the maximum force required to puncture the stalk rind.

For ECB data, plants were infested at whorl stage (8–10 leaves) or anthesis with approximately 120 live, neonate larvae. Egg cases were obtained from the USDA-ARS Corn Insect and Crop Genetics Research Unit, Ames, IA. Egg cases were enclosed in sterilized glass jars in the dark and the hatched larvae were gathered by corncob grits. An infestor (Product no. F9050, Bio-Serv, Frenchtown, NJ) was used to transfer larvae to the plant. To simulate a first-generation ECB infestation, larvae were placed in the whorl at the whorl stage for the first six plants in the third row of each plot. To simulate second-generation ECB infestations, larvae were deposited in the ear zone and in the node axil above the primary ear down to the node axil below the bottom ear at anthesis for the last 11 plants in the third row of each plot.

First-generation ECB damage was visually rated with a nine-point scale for field damage ratings, with nine being most susceptible (Guthrie et al., 1960). First-generation damage was rated on the first five plants in the third row, and second-generation damage was evaluated on the last 10 plants in the third row. A larger number of plants was used for second-generation damage because it is the more economically important generation and the primary focus of this experiment. Second-generation ECB damage was evaluated by splitting stalks from the ground to the internode above the primary ear with linoleum knives, counting the number of tunnels, and visually estimating the length of the tunnels.

With the approach of Bergvinson et al. (1994), we manufac-

¹ Mention of a trademark or proprietary product does not constitute a guarantee, warranty, or recommendation of the product by the U.S. Department of Agriculture or the University of Missouri and does not imply its approval to the exclusion of other products that may also be suitable.

Table 1. Trait means for Missouri Second Cycle Stiff Stalk Synthetic low (LRP) and high (HRP) selections and susceptible and resistant checks evaluated with three replications. Number of environments are shown parenthetically beside the trait name.[†]

Entries	Agronomic traits			ECB traits		
	Grain yield (4)	Stalk lodging (4)	RPR (6)	1ECB (6)	Tunnel no. (6)	Tunnel length (6)
	t ha ⁻¹	%	kg plant ⁻¹	1-9 scale [‡]		cm
C0\$	7.73	47.0	3.55	3.2	2.0	6.6
C3LRP	7.42	54.0	2.99	3.3	2.0	6.3
C6LRP	6.12	61.1	2.46	3.4	2.4	7.3
C9LRP	5.99	64.9	2.14	3.3	3.5	12.3
C12LRP	5.37	62.8	2.00	3.1	3.3	12.8
C3HRP	7.29	30.1	4.49	3.3	1.5	5.3
C6HRP	6.51	20.7	5.20	3.0	1.5	5.3
C9HRP	6.31	10.1	6.67	3.5	1.4	4.5
C12HRP	5.55	10.3	8.52	3.4	1.8	6.3
Wf9 × W182E	7.94	25.4	3.51	3.9	2.8	9.1
Pioneer Brand 3184	8.16	13.3	4.47	2.3	0.4	1.2
Mean	6.84	37.2	4.13	3.3	2.1	7.0
LSD 0.05¶	0.57	7.1	0.25	0.3	0.4	1.4
CV%#	14.3	31.9	8.0	18.5	35.8	40.4

Entries	Leaf traits		Stalk component traits ^{††}			
	LPR1 (6)	LPR2 (6)	Crude fiber (6)	Cellulose (6)	Lignin (6)	Silica (6)
	g plant ⁻¹		% of stalk dry matter			
C0\$	151.3	152.1	42.24	43.21	8.17	1.10
C3LRP	144.4	146.3	41.48	43.03	7.41	1.14
C6LRP	146.3	152.5	40.15	41.72	6.95	1.17
C9LRP	143.3	154.5	41.04	42.48	6.71	1.15
C12LRP	152.3	157.5	40.32	41.12	7.97	1.36
C3HRP	154.4	142.5	43.82	44.61	8.79	1.13
C6HRP	150.5	144.8	43.71	45.12	8.70	1.06
C9HRP	150.3	148.7	45.62	44.73	8.85	0.98
C12HRP	158.7	148.2	44.60	44.34	9.40	0.90
Wf9 × W182E	156.5	147.4	41.74	43.04	8.01	1.09
Pioneer Brand 3184	153.6	162.3	43.26	44.14	9.15	1.06
Mean	151.1	150.8	42.52	43.40	8.19	1.10
LSD 0.05¶	4.0	5.9	1.27	1.13	0.88	0.14
CV%#	6.5	6.4	3.7	3.2	13.1	15.5

[†] ECB, European corn borer; 1ECB, first-generation European corn borer damage; LPR1, leaf penetrometer resistance at the whorl stage of plant development; LPR2, leaf penetrometer resistance at anthesis; RPR, rind penetrometer resistance.

[‡] Guthrie et al. (1960) 9-point ECB leaf-feeding damage scale wherein 1 represents no damage and 9 represents extensive damage.

^{\$} Mean of duplicate entries.

[¶] LSD 0.05 based on the environments × entries mean square.

[#] On the basis of the pooled error for agronomic, ECB, and leaf traits, and on the environments × entries interaction for stalk component traits.

^{††} Stalk samples from three replications were combined within environments by entry.

tured a leaf penetrometer to measure leaf toughness. An Accu-Force Cadet digital force gage, 0- to 500-g capacity, powered by a 9-V alkaline battery (Ametek, Hunter Division, Hatfield, PA) (Fig. 2) was attached to a ball-bearing slide and a 76-cm vertical arm that moved up and down at consistent speed. The vertical arm was attached to a short rotating arm powered by a 115-V, RI0091 reduction-drive motor (Dayton, Chicago, IL). This column holding the penetrometer and drive assembly was mounted onto a 36- by 41- by 2.5-cm metal base. A hole just larger than the size of the blunt-tipped needle (0.8 mm) was drilled into a positionable anvil to allow alignment and for penetration of the needle through the leaf. An up-down-up measurement cycle required approximately 2 s. Although penetration force was displayed to one-tenth gram, measurements were recorded to the nearest whole gram.

Leaf penetration resistance was recorded at the whorl stage and at tasseling in the fourth row of each plot. A leaf section was taken from the third leaf out from the whorl center or the primary ear leaf for each sample date, respectively. Each leaf sampled was trimmed to approximately a 15-cm section halfway between the base and tip of the leaf. Ten leaves were taken from separate plants in each plot, labeled, and placed in an ice chest for preservation during transport back to the laboratory in Columbia. For each leaf, two punches between the ribs were recorded for a total of 20 observations per plot.

Stalk samples from the fifth row in each plot were used for chemical analysis of crude fiber, cellulose, lignin, and silica.

The complete internode below the primary ear was harvested from 10 competitive plants (plants with adjacent plants present) and air dried for at least three months. Then, with a double-bladed saw, a 5.1-cm section of stalk was cut from the center of the internode. These sections were ground with a Thomas-Wiley mill (Model 4, Arthur H. Thomas, Philadelphia, PA) to pass through a 1-mm mesh screen. The ground



Fig. 1. Measuring rind penetrometer resistance with the modified electronic rind penetrometer in the middle of the internode below the primary ear attachment node.

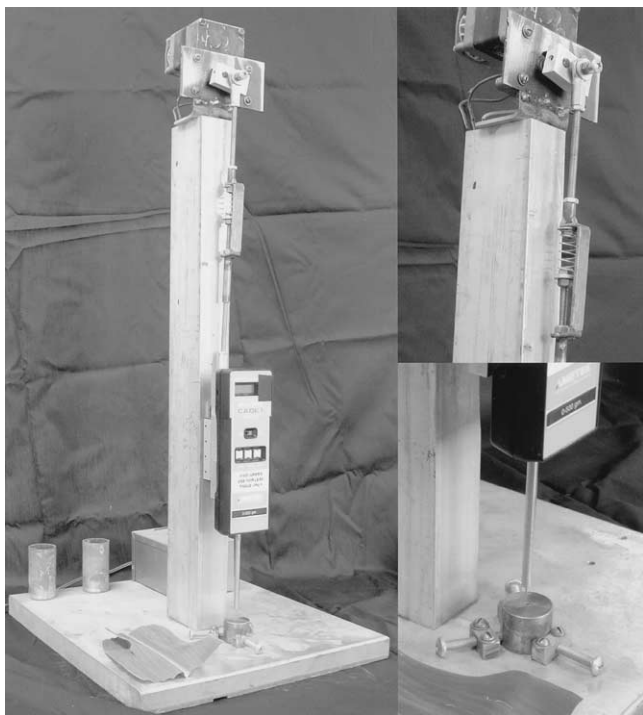


Fig. 2. Modified electronic leaf penetrometer measuring the force necessary to puncture the leaf between ribs.

stalk was well mixed and placed into labeled plastic bags. To reduce the number of samples for analysis, ground stalks from the three replications at each location for each entry were mixed together for a total of 12 samples from each of the six locations. Stalk samples were analyzed by the Missouri Agricultural Experiment Station Chemical Laboratories. All compounds were analyzed with the American Association of Analytical Chemists (AOAC) official methods of analysis for crude fiber, 978.10; cellulose, 973.18 (A–D); lignin, 973.18 (A–D); and silicates in plant tissue, 920.08 (AOAC, 1995).

Statistical Analysis System (SAS) procedures, version 8 (SAS Institute, 2001), were used to analyze the data. Grain yield data were adjusted to 155 g kg⁻¹ moisture. Combined ANOVAs were performed across environments. Sources of variation included environments, replications within environments, entries, checks vs. all cycle entries, among checks, among all cycle entries, duplicates of cycle 0, and among cycles for the agronomic and ECB traits. Entries and all of its partitions were considered fixed effects in the ANOVA model and environments were considered random effects.

Least squares analysis (Eberhart, 1964) was used to partition the among-cycles variation. Sums of squares were obtained for linear and quadratic responses simultaneously fit in both the high and low directions of selection. The highest order significant model for each trait was chosen as the best description of response to selection. The R^2 statistic for the response to selection fit applies to the simultaneous fit in both the high and low directions. The sum of squares for two linear regressions was partitioned into an average response (average linear), showing whether selection response was greater in one direction than the other, and a difference between the two linear regressions. The combined ANOVA and least squares analysis for the ground stalk sample traits were performed similar to those for agronomic and ECB traits, but there was no pooled error term. Instead, all sources of variation were tested against the environment \times entries interaction.

RESULTS AND DISCUSSION

Rind penetrometer resistance selection was effective at separating the original population of MoSCSSS into two significantly different subpopulations. There were significant differences between the subpopulations for grain yield, stalk lodging percentage, rind penetrometer resistance, ECB stalk tunnel number and tunnel length, leaf penetrometer resistance at both whorl-stage and anthesis, and stalk composition, including crude fiber, cellulose, lignin, and silica.

Grain yield decreased in both directions of selection and showed significant differences from the original population. (Table 1, Fig. 3A). There were highly significant ($P < 0.01$) differences among environments and entries in the combined ANOVA (not shown). The environment \times entries interaction mean square was not significant, indicating consistency in genotype performance across environments. Grain yield response to selection was described by a linear fit ($R^2 = 0.21$ for the simultaneous fit in the high and low directions of selection, Fig. 3A). The average decrease resulting from selection was 2.5% cycle⁻¹ with decreases occurring for both directions of selection. Chesang-Chumo (1993) found no significant differences in grain yield because of rind penetrometer resistance selection of MoSCSSS through Cycle 5 in the high and low directions. However, there was a nonsignificant, negative correlation between grain yield and rind penetrometer resistance (Table 2). As selection continued in both directions, grain yield decreased and there was a significant grain yield reduction by cycle 6 (Table 1). This may be associated with the increased stalk lodging percentage in the low direction of selection and the reallocation of carbohydrates and other nutrients to the stalk instead of the grain in the high direction of selection. An effect from inbreeding depression might have contributed to grain yield reduction. However, with approximately 120 plants pollinated for recombination in each cycle, that potential effect should have been minimized.

There were no significant differences in root lodging percentage (data not shown). Stalk lodging percentage, however, was significantly different and showed greater and wider ranges of values than for root lodging (Table 1). There were significant ($P < 0.05$) differences among environments and highly significant differences among entries. The response to selection was described by a quadratic fit ($R^2 = 0.55$, Fig. 3B), which was significant for both quadratic regressions. There was a clear separation in the high and low populations with a significantly greater response (average response more toward reduced stalk lodging) for the high direction of selection for stalk lodging percentage. This indicates that selection for rind penetrometer resistance can significantly affect stalk lodging percentage, increasing or decreasing its occurrence, and rind penetrometer resistance selection can provide resistance to stalk lodging. Stalk lodging percentage was negatively correlated with rind penetrometer resistance, crude fiber, cellulose, and lignin, and positively correlated with second-generation ECB tunnel number and length, and silica concentration (Table 2). Response to selection was consistent up to cycle

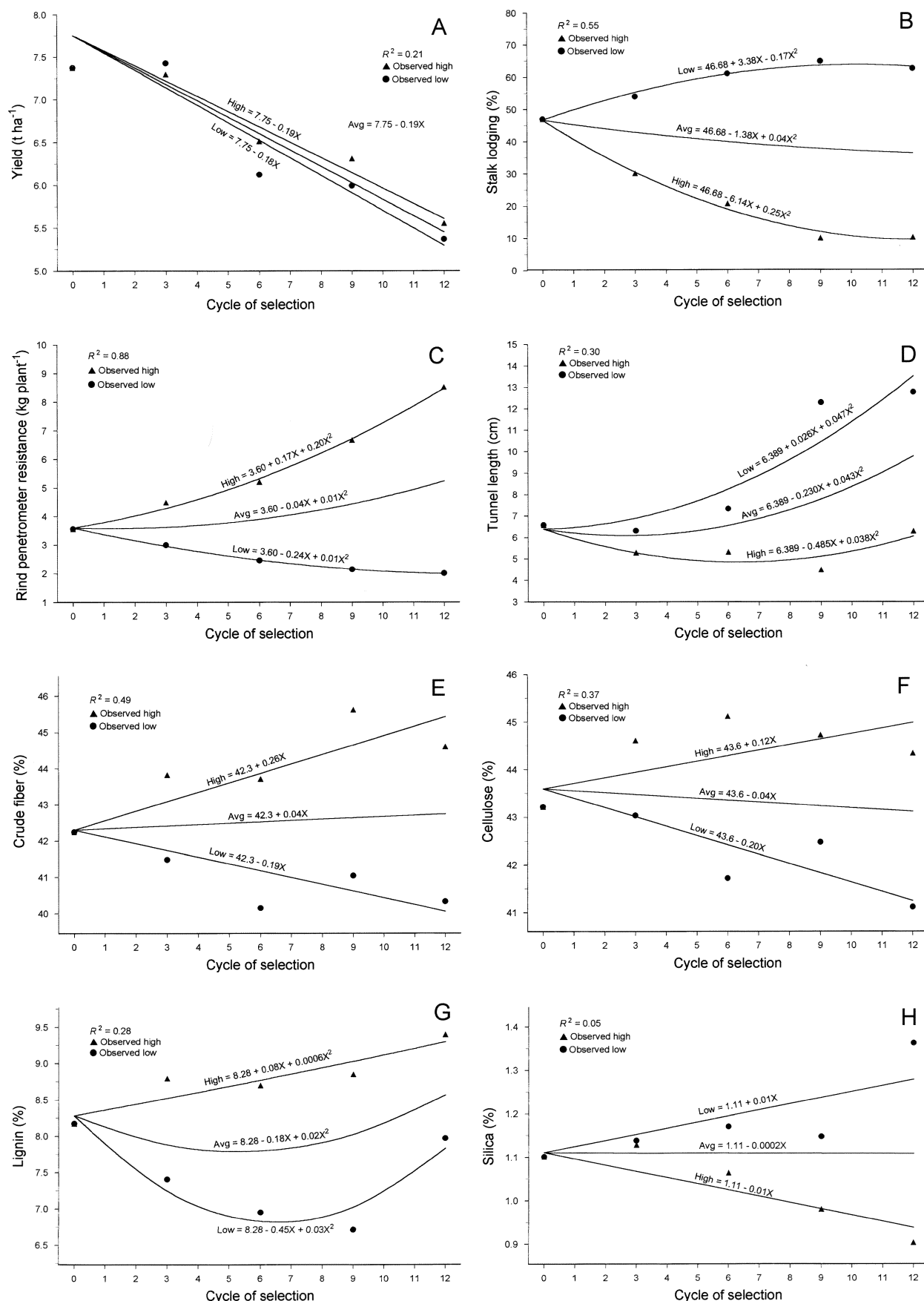


Fig. 3. Predicted responses to bidirectional rind penetrometer resistance selection for (A) grain yield (four environments), (B) stalk lodging (four environments), (C) rind penetrometer resistance (six environments), (D) second-generation European corn borer damage (six environments), (E) percentage crude fiber in the stalk (six environments), (F) percentage cellulose in the stalk (six environments), (G) percentage lignin in the stalk (six environments), and (H) percentage silica in the stalk (six environments).

Table 2. Correlations among characters of Missouri Second Cycle Stiff Stalk Synthetic selected cycles from data combined across environments. $N = 9$.†

Parameters	Stalk lodging	RPR	IECB	Tunnel number	Tunnel length	LRP1	LRP2	Crude fiber	Cellulose	Lignin	Silica
Grain yield	0.01	-0.14	-0.07	-0.47	-0.52	-0.19	-0.51	0.10	0.31	-0.01	-0.08
Stalk lodging		-0.95**	-0.22	0.81**	0.74*	-0.66	0.64	-0.97**	-0.90**	-0.90**	0.83**
RPR			0.34	-0.70*	-0.64	0.70*	-0.52	0.90**	0.78*	0.86**	-0.89**
IECB				-0.18	-0.28	-0.02	-0.07	0.29	0.08	0.00	-0.47
Tunnel number					0.98**	-0.43	0.85**	-0.79*	-0.85**	-0.71*	0.68*
Tunnel length						-0.31	0.83**	-0.72*	-0.79*	-0.58	0.70*
LRP1							-0.26	0.58	0.43	0.89**	-0.38
LRP2								-0.65	-0.84**	-0.53	0.55
Crude fiber									0.92**	0.85**	-0.82**
Cellulose										0.75*	-0.79**
Lignin											-0.59

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† IECB, first-generation European corn borer damage; LRP1, leaf penetrometer resistance at the whorl stage of plant development; LRP2, leaf penetrometer resistance at anthesis; RPR, rind penetrometer resistance.

9 in both directions; however, in Cycle 12 in the high and low directions, there was a slight increase and decrease in stalk lodging, respectively. Of all agronomic traits, stalk lodging is most likely the reason for grain yield loss in the low direction of selection with up to 64.9% lodging at Cycle 9.

Rind penetrometer resistance showed highly significant differences because of environments and entries (not shown). Rind penetrometer resistance results showed an excellent fit of a quadratic model ($R^2 = 0.88$, Fig. 3C) and there was a significantly greater response to selection in the high direction of selection than the low direction, most likely because of reduced genetic variance in the low direction of selection. Rind penetrometer resistance was negatively correlated with second-generation ECB tunnel number and silica, and was correlated with leaf penetrometer resistance at whorl-stage, crude fiber, cellulose, and lignin (Table 2). There was also a highly significant difference between the two ECB checks (Table 1). The observed mean for the susceptible check was 3.5 kg plant⁻¹ and the resistant check mean was 4.5 kg plant⁻¹, suggesting that ECB resistance might be due, at least partially, to stalk strength.

Rind penetrometer resistance, which was highly correlated with stalk lodging ($r = -0.95$, $P < 0.01$, Table 2), accounted for the decrease in stalk lodging in the high direction of selection; however, harvestable grain yield still decreased in this direction of selection. Because the plants were still standing, this grain yield decrease was most likely due to the reallocation of photosynthates within the plant. Possibly because of the focus on stalk strength selection, much of the plant's photosynthates were redirected to the stalk instead of to the grain, resulting in decreased grain yield. In other words, sink strength of the stalk was increased. It is also possible that inbreeding depression might have contributed to grain yield reduction. However, with approximately 120 plants pollinated for recombination in each cycle, that potential effect should have been minimized.

There were no significant differences in the first-generation ECB damage because of rind penetrometer resistance selection in either the low or high directions, indicating that the factors that affect rind penetrometer resistance and stalk strength do not play a role in first-generation ECB damage. There were significant differ-

ences due to rind penetrometer resistance selection in both number of tunnels and tunnel length for second-generation ECB damage. Response to selection for tunnel length was described by a quadratic fit ($R^2 = 0.30$, Fig. 3D) and the response to selection was greater in the low direction of selection, with increases both in tunnel number (data not shown) and length. The high direction of selection showed the greatest resistance change occurred between Cycles 0 and 3 for tunnel length. A similar response occurred for tunnel number.

Response for leaf penetrometer resistance at the whorl stage was quadratic, although a very poor fit ($R^2 = 0.07$, data not shown). The response to selection was greater in the high direction of selection, indicating there was an increase in leaf penetrometer resistance with an increase of rind penetrometer resistance. Leaf penetrometer resistance at whorl stage was correlated with rind penetrometer resistance and lignin concentrations (Table 2). At anthesis, leaf penetrometer resistance showed a linear response to selection, but again, a very poor fit to the model ($R^2 = 0.07$, data not shown). The response to selection was greater in the low direction of selection, and the responses to selection direction were reversed from the whorl stage, for example, the high direction of selection had lowered leaf penetrometer resistance values. No biological explanation for this observation was apparent. Leaf penetrometer resistance at anthesis was correlated with second-generation ECB damage.

The chemical analysis of stalk strengthening compounds showed that there were significant changes occurring in the stalk because of rind penetrometer resistance selection. Crude fiber percentage response to selection was described by a linear fit ($R^2 = 0.49$, Fig. 3E) and was of equal magnitude (nonsignificant average linear response) and in opposite directions, with selection for high rind penetrometer resistance increasing crude fiber percentage. Highly significant correlations with crude fiber were found for stalk lodging percentage and rind penetrometer resistance (Table 2). Significant correlations with crude fiber were found for both tunnel number and tunnel length (Table 2). Cellulose percentage response to selection was described by a linear fit ($R^2 = 0.37$, Fig. 3F) with responses opposite in direction, but equally strong. Selection for high rind penetrometer resistance increased cellulose percentage. Highly signifi-

cant correlations were found with stalk lodging percentage, tunnel number, leaf penetrometer resistance at anthesis, and crude fiber percentage (Table 2). Significant correlations with cellulose were found for rind penetrometer resistance and tunnel length (Table 2). Lignin percentage response to selection was described by a quadratic fit ($R^2 = 0.28$, Fig. 3G) and there was a pronounced increase in lignin concentration in Cycle 12 in the low direction of selection after linearly decreasing through Cycle 9, resulting in an overall quadratic response. Response in the high direction had a very small quadratic contribution making the appearance linear. Highly significant correlations for lignin occurred with stalk lodging percentage and rind penetrometer resistance, and a significant correlation was found with tunnel number (Table 2). Silica percentage response to selection was very poor, but had a significant linear fit ($R^2 = 0.05$, Fig. 3H). Responses were equivalent and opposite in direction for the high and low directions of selection with the low direction of selection having increased silica content. Silica was highly correlated with stalk lodging percentage and rind penetrometer resistance; however, silica was inversely related to these traits when compared with the other stalk traits analyzed (Table 2). Silica was also significantly and positively correlated with second-generation ECB damage, which differs from results of Coors (1988) who found significant negative correlations between silica content in the leaf sheath and collar tissues, and ECB damage. We can offer no explanation of this difference except for the differing germplasm being evaluated.

Each of the stalk compounds was correlated with ECB tunnel number and highly correlated with stalk lodging percentage (Table 2). Their correlation with rind penetrometer resistance suggests that selection may be largely based on stalk strengthening compounds. Resistance to stalk lodging and second-generation ECB was most likely because of these structural changes occurring in the stalk. These results support findings by Coors (1988), who found that neutral detergent fiber and lignin were significantly associated with ECB resistance. However, Coors (1988) also found significant negative correlations between silica content and ECB damage. This experiment does not support his findings, but instead, ECB damage increased with increasing silica content in this experiment.

The significant correlations found between stalk lodging, rind penetrometer resistance, second-generation ECB damage, and stalk composition showed that these traits are closely related to each other and that the adjustments in stalk compositions most likely influenced the responses of the other traits. Although silica responses were reversed from the other stalk strengthening compounds analyzed, even at Cycle 12 in the low direction, it only comprised 1.36% of the stalk. Stalk strength in MoSCSSS most likely came from cellulose and crude fiber, comprising up to 50% of the stalk. The increase in these compounds most likely accounted for the correlations with stalk lodging and second-generation

ECB tunnel number, reaffirming the importance of stalk composition in deterring these problems.

Selection for rind penetrometer resistance was successful at separating a single MoSCSSS population into high and low strains. With an increase in rind penetrometer resistance, there was a decrease in second-generation ECB tunnel number and lengths. Also, there were increases and decreases in the stalk components, as determined by the high or low direction of selection, respectively, with the exception of silica. This indicated that these components play a role in strengthening of the stalk and may contribute to less feeding by second-generation ECB. However, the Cycle 12 observed values for some traits, including stalk lodging percentage, number of tunnels, tunnel length, leaf penetrometer resistance at the whorl-stage, and lignin reversed direction from that which would be predicted based on Cycles 0 through 9. The differences were usually negligible and could be a result of random error. Use of selection for rind penetrometer resistance cannot now be recommended for reduction of second-generation ECB damage. A future evaluation involving additional selection and different germplasm selected for stalk crushing strength is planned.

REFERENCES

- AOAC. 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Gaithersburg, MD.
- Bergvinson, D.J., J.T. Arnason, R.I. Hamilton, J.A. Mihm, and D.C. Jewell. 1994. Determining leaf toughness and its role in maize resistance to the European corn borer (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 87:1743-1748.
- Buendgen, M.R., J.G. Coors, A.W. Grombacher, and W.A. Russell. 1990. European corn borer resistance and cell wall composition of three maize populations. *Crop Sci.* 30:505-510.
- Chesang-Chumo, J. 1993. Direct and correlated responses to divergent selection for rind penetrometer resistance in MoSCSSS maize synthetic (*Zea mays*). Ph.D. thesis. Univ. of Missouri, Columbia.
- Coors, J.G. 1988. Nutritional factors related to European corn borer resistance in maize. p. 76-88. *In* D. Wilkinson (ed.) Proc. of the Annual Corn and Sorghum Res. Conf., 42nd, Chicago, IL. 8-9 Dec. 1988. American Seed Trade Association, Washington, DC.
- Eberhart, S.A. 1964. Least squares method for comparing progress among recurrent selection methods. *Crop Sci.* 4:230-231.
- Gerdes, J.T., C.F. Behr, J.G. Coors, and W.F. Tracy. 1993. Compilation of North American maize breeding germplasm. CSSA, Madison, WI.
- Guthrie, W.D., F.F. Dicke, and C.R. Nieswander. 1960. Leaf and sheath feeding resistance to the European corn borer in eight inbred lines of dent corn. *Res. Bull.* 860. Ohio Agric. Exp. Stn., Wooster.
- Jarvis, J.L., R.L. Clark, and W.D. Guthrie. 1982. Effect of second generation European corn borer on resistance of maize to *Diplodia maydis*. *Phytopathology* 72:1149-1152.
- Klenke, J.R., W.A. Russell, and W.D. Guthrie. 1986. Grain yield reduction caused by second generation European corn borer in BS9 corn synthetic. *Crop Sci.* 26:859-863.
- Masole, H. 1993. Evaluation of high and low divergent rind penetrometer resistance selection at three plant densities in maize. M.Sc. thesis. Univ. of Missouri, Columbia.
- SAS Institute. 2001. The SAS program. Release 8.1 ed. SAS Inst., Cary, NC.
- Sibale, E.M., L.L. Darrah, and M.S. Zuber. 1992. Comparison of two rind penetrometers for measurement of stalk strength in maize. *Maydica* 37:111-114.
- Zuber, M.S., T.R. Colbert, and L.L. Darrah. 1980. Effect of recurrent selection for crushing strength on several stalk components in maize. *Crop Sci.* 20:711-717.
- Zuber, M.S., and M.S. Kang. 1978. Corn lodging slowed by sturdier stalks. *Crops Soils* 30:13-15.